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Effect of 4,4-dimethylmorpholinium chloride and allied compounds on growth of the lettuce hypocotyl

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Abstract

4,4-dimethylmorpholinium chloride (DMMC) at 1 mM concentration inhibited growth of lettuce hypocotyl by about 50%, the effect being reversed by higher doses of gibberellic acid. Structural analogues of DMMC, 4,4-dimethyl-2-oxo-morpholinium chloride (DMOMC) and 4,4-diethyl-2-oxo-morpholinium chloride (DEOMC) did not effect growth of the hypocotyl. All three compounds at the concentration of 10 mM inhibited growth of the radicle.

INTRODUCTION

4,4-dimethylmorpholinium chloride (DMMC), 4,4-dimethyl-2-oxo--morpholinium chloride (DMOMC) and 4,4-diethyl-2-oxo-morpholinium chloride (DEOMC) have intentionally been synthesized as plant growth retardants (W i t e k et al. 1967) and originally encoded as RW-3, RW-13 and RW-14, respectively (Fig. 1). Screening tests revealed that the compounds more or less selectively retard growth of cereal and vegetable plants, DMMC affecting the broadest spectrum of different plant species

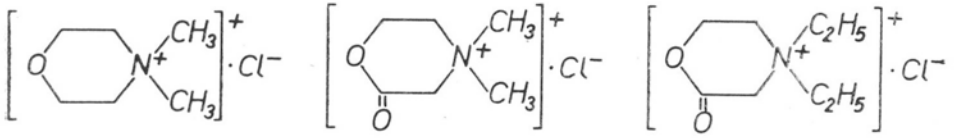

Fig. 1. Chemical structure of 4,4-dimethylmorpholinium chloride (DMMC), 4,4-dimethyl-2-oxo-morpholinium chloride (DMOMC) and 4,4-diethyl-2-oxo-morpholinium chloride (DEOMC)

(Krawiec 1974; Krawiec et al. 1973). The compounds, depending on the mode of application and environmental conditions, can stimulate growth of leguminous plants (Krawiec 1973). DMMC on the contrary to DMOMC and DEOMC can stimulate growth of ryegrass (Żurawski et al. 1973). It enhances also the resistance of lettuce to dry tipburn, and to wet rot caused by *Botrytis* sp. (Borkowski 1975).

It has recently been reported that DMMC retards growth of *Spirodela oligorrhiza*, the effect being reduced by gibberellic acid (GA_3) and benzyladenine (Knypl et al. 1976). This effect suggested that this compound possibly interfered with the biosynthesis or interconversions of gibberellins in plants (cf. Lang 1970). The aim of this study was to test how DMMC and related compounds affect growth of the lettuce hypocotyl.

MATERIAL AND METHODS

The lettuce hypocotyl biotest was adapted from Hradilík (1967) and Okagami (1971). Seeds of lettuce, *Lactuca sativa* L. cv. Królowa Majowych, were pre-germinated for 24 h in darkness at 20°C. The germinated seeds were transferred to 7 cm Petri dishes lined with 2 discs of Whatman No. 2 blotting paper moistened with 2.5 ml of distilled water (the control) or 2.5 ml aliquots of solutions of growth retardants alone or in combination with gibberellin. After 4 days of growth under continuous illumination at 24°C the length of the hypocotyl was measured. Of 15 seedlings in each Petri dish, two extreme ones were discarded. The experiments were repeated twice or thrice, with two replications in each run.

Fluorescent tubes "Flora LF 40W" (Unitra-Polam) were used to illuminate the seedlings (1.1 Klx at the tissue level). Low light intensity decreased the sensitivity of this bioassay to gibberellin (cf. Okagami 1971), and allowed experiments with growth inhibitors to be carried out because of more vigorous growth of the control plants.

Solutions of all compounds were freshly prepared and brought to pH 6.0 by means of NaOH.

RESULTS

DMMC at the concentration of 1 mM decreased the growth of lettuce hypocotyl by about 50% in comparison with the water treated control, without affecting the radicle growth. This compound at higher concentrations markedly inhibited growth of the radicle, but the degree of inhibition of hypocotyl growth was not furtherly potentiated (Table 1).

DMOMC and DEOMC even at 10 mM concentration did not significantly affect growth of the hypocotyl whereas growth of the radicle was inhibited (Table 1).

Fig. 2 shows that the inhibitory effect of DMMC (1 mM) on growth of lettuce hypocotyl was partially reversed by 10^{-6} M, GA_3 and completely nullified by the highest dose of GA_3, 10^{-5} M.

Table 1

Comparative effects of DMMC, DMOMC and DEOMC on growth of lettuce seedlings

Measurements were made after 4 days of growth in light. Data followed by unlike postscripts within each column differ significantly at the 1 % probability level. DMMC at 10 mM inhibited greening of the cotyledons.

Treatment	Lenght, mm	
	Hypocotyl	Radicle
H_2O	$7.4^{a,c}$	30.8^{a}
DMMC, 1 mM	3.3^{b}	29.4^{a}
DMMC, 5 mM	4.0^{b}	23.5^{c}
DMMC, 10 mM	4.0^{b}	13.9^{b}
DMOMC, 5 mM	6.8^{a}	18.9^{d}
DMOMC, 10 mM	6.7^{a}	$21.2^{c,d}$
DEOMC, 5 mM	$7.4^{a,c}$	33.4^{a}
DEOMC, 10 mM	8.1^{c}	17.7^{d}

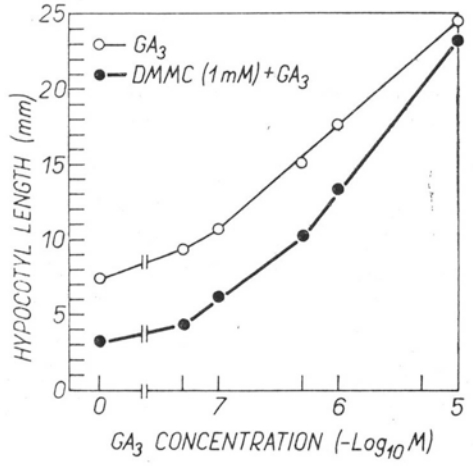

Fig. 2. Reversal of the retarding effect of DMMC on growth of lettuce hypocotyl by higher concentrations of gibberellic acid. Length of the hypocotyl was measured after 4 days of cultivation in solutions of GA_3 (open circles) or solutions of GA_3 supplemented with 1 mM of DMMC (black circles). 0 = the water or DMMC treated controls (open and black circles, respectively).

DISCUSSION

Some of the growth retardants exert a dwarfing effect on plants because they inhibit the biosynthesis of gibberellins (cf. Lang 1970). One of the methods of testing the possible interaction of growth retardants with endogenous gibberellins is to apply varying amounts of GA to plants in the presence of a fixed amount of the retardant. If the growth inhibition due to the retardant is completely overcome by the phytohormone, one can infer that the retardant in question interferes with the metabolism of the gibberellins (Lockhart 1962; Moore 1967).

Figure 2 shows that the retarding effect of DMMC on growth of lettuce hypocotyl can be counteracted by higher doses of GA_3. However, the effective concentration of GA_3 (0.01 mM) is rather very high. This seems to indicate that the retarding effect of DMMC on hypocotyl growth is not solely due to the inhibition of biosynthesis of gibberellins.

Previous experiments revealed that the retarding effect of DMMC on growth of cucumber hypocotyl could be counteracted by either GA_3 or auxin (Knypl 1977). The same holds true for 2-chloroethyl--trimethylammonium chloride (CCC), but not for other growth retardants (Moore 1967). Recently it was also found that DMMC can induce *de novo* synthesis or enhance activity of some isoenzymes of ribonuclease and phosphatase in *Spirodela oligorrhiza* (Knypl 1976). Both DMMC and CCC at higher concentrations inhibit chlorophyll synthesis (Knypl et al 1976).

It seems that the mechanism of action of DMMC is as complicated as the mechanism of action of CCC. It is known that CCC can directly affect the synthesis and breakdown of protein in lettuce (Knypl and Chylinska 1972). The same possibly hold true for DMMC.

It cannot be explained, as yet, why DMOMC and DEOMC, the compounds so closely related to DMMC, are not active as the inhibitors of growth of lettuce hypocotyl. Borkowski (1975) in prolonged greenhouse experiments has found that DMOMC and DEOMC either not affected or slightly stimulated growth of lettuce plants, whereas DMMC was as active growth retardant for lettuce as CCC. Heterocyclic ring of DMOMC and DEOMC can be opened to form betains (Witek and Oświęcimska, personal communication). If this reaction takes place *in vivo*, the formation of betains can explain the lack of the inhibitory effect of DMOMC and DEOMC on growth of the lettuce hypocotyl.

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Wpływ chloorku 4,4-dwumetylomorfoliniowego i związków pokrewnych na wzrost hypokotyli sałaty

Streszczenie

Chlorek 4,4-dwumetylomorfoliniowy (DMMC) w stężeniu 1 mM hamuje o około 50% wzrost wydłużeniowy hypokotyli siewek sałaty, przy czym efekt ten jest całkowicie odwracany przez giberelinę. Strukturalne analogi DMMC: chlorek 4,4-dwumetylo-2-oksomorfoliniowy i chlorek 4,4-dwuetylo-2-oksomorfoliniowy nie wpływają na wzrost hypokotyli. Wszystkie wymienione substancje w stężeniu 10 mM hamują wzrost korzeni.